Novel 5-Hydroxytryptamine 4 (5-HT$_4$) Receptor Agonists. Synthesis and Gastroprokinetic Activity of 4-Amino-*N*-[2-(1-aminocycloalkan-1-yl)ethyl]-5-chloro-2-methoxybenzamides

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A novel series of 4-amino-*N*-[2-(1-aminocycloalkan-1-yl)ethyl]-5-chloro-2-methoxybenzamide derivatives (1), which had amines conformationally restricted due to the effect of repulsion by neighboring substituents, were prepared and evaluated for 5-hydroxytryptamine 4 (5-HT$_4$) agonistic activities by using the contraction of longitudinal muscle myenteric plexus (LMMP) of guinea pig ileum. One of the most potent compounds in this series was 4-amino-5-chloro-*N*-[2-(1-dimethylamino-1-cyclohexyl)ethyl]-2-methoxybenzamide (1c, YM-47813) with an EC$_{50}$ value of 1.0 μM on LMMP. This compound effectively enhanced gastric motility and gastric emptying in conscious dogs by oral administration (1—3 mg/kg).

Key words 5-HT$_4$ agonist; structure–activity relationship; benzamide

Serotonin (5-hydroxytryptamine, 5-HT) is widely distributed in the central nervous, gastrointestinal, and cardiovascular systems as a neurotransmitter, neuromodulator and hormone. Currently, 5-HT receptors are classified into at least seven distinct groups (5-HT$_1$—5-HT$_7$).[1] The 5-HT$_4$ receptor has been found in the central nervous system as a receptor positively linked to adenylate cyclase (Dumuis *et al.*, 1988),[2] and was subsequently reported to be distributed in guinea pig hippocampus (Bockaert *et al.*, 1990),[3] ileum (Craig and Clarke, 1990, Eglen *et al.*, 1990),[4,5] colon (Elswood *et al.*, 1991),[6] and rat esophagus (Baxter *et al.*, 1991).[7] Two types of 5-HT$_4$ receptors were cloned from rat brain and shown to be G-protein-coupled seven transmembrane receptors.[8] In the gastrointestinal system, the 5-HT$_4$ receptor is distributed from the esophagus to the colon, and is especially closely related with regulation of gastrointestinal motility.[9] This gastroprokinetic activity is ascribed to a release of acetylcholine by stimulation of the 5-HT$_4$ receptor in parasympathetic ganglia.[9] Recently, the 5-HT$_4$ receptor was reported to be related to secretion and motility in the colon and the possibility of regulation of the colon was investigated.[10] Thus, 5-HT$_4$ agonists are expected to be effective for treatment of gastrointestinal dysfunctions such as diarrhea, constipation, gastroparesis, ileus, reflux esophagitis, and pseudo-obstructions.

Reported 5-HT$_4$ agonists are roughly divided into two groups on the basis of chemical structure. The first type are 5-HT derivatives[11] and the other are the benzamide (or other arylcarboxamide) derivatives.[9] Both types have basic amines on the side chain as an interaction part. Amongst benzamide derivatives, renzapride[12] and zacopride[13] were originally reported as 5-HT$_3$ antagonists and then subsequently found to have gastroprokinetic activities

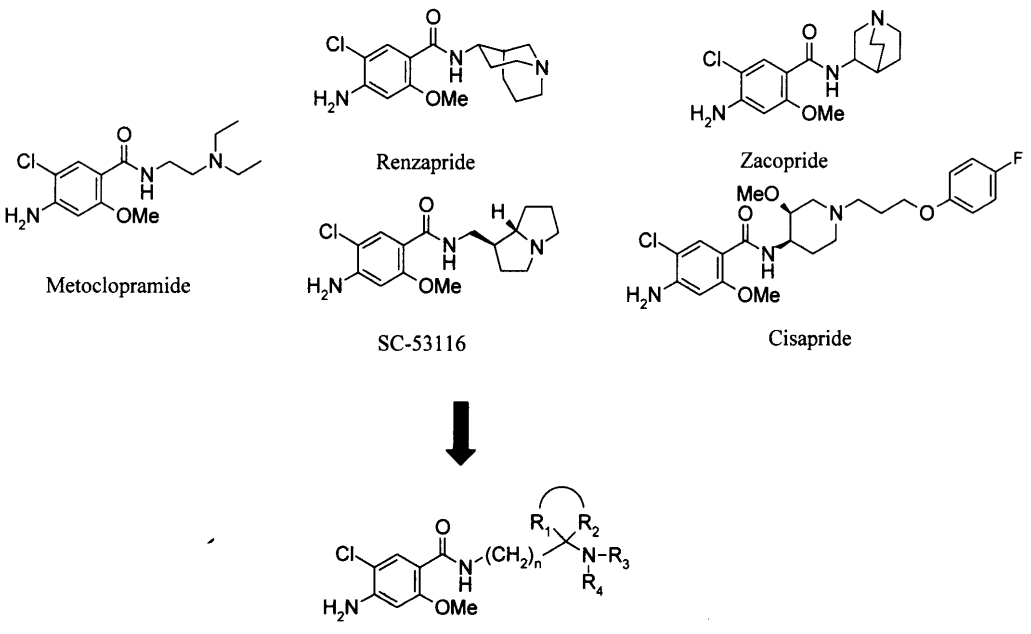

Chart 1

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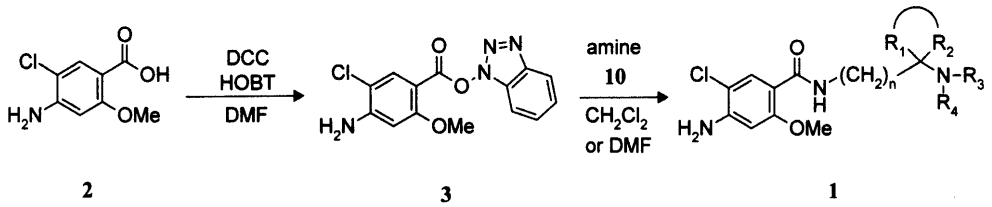
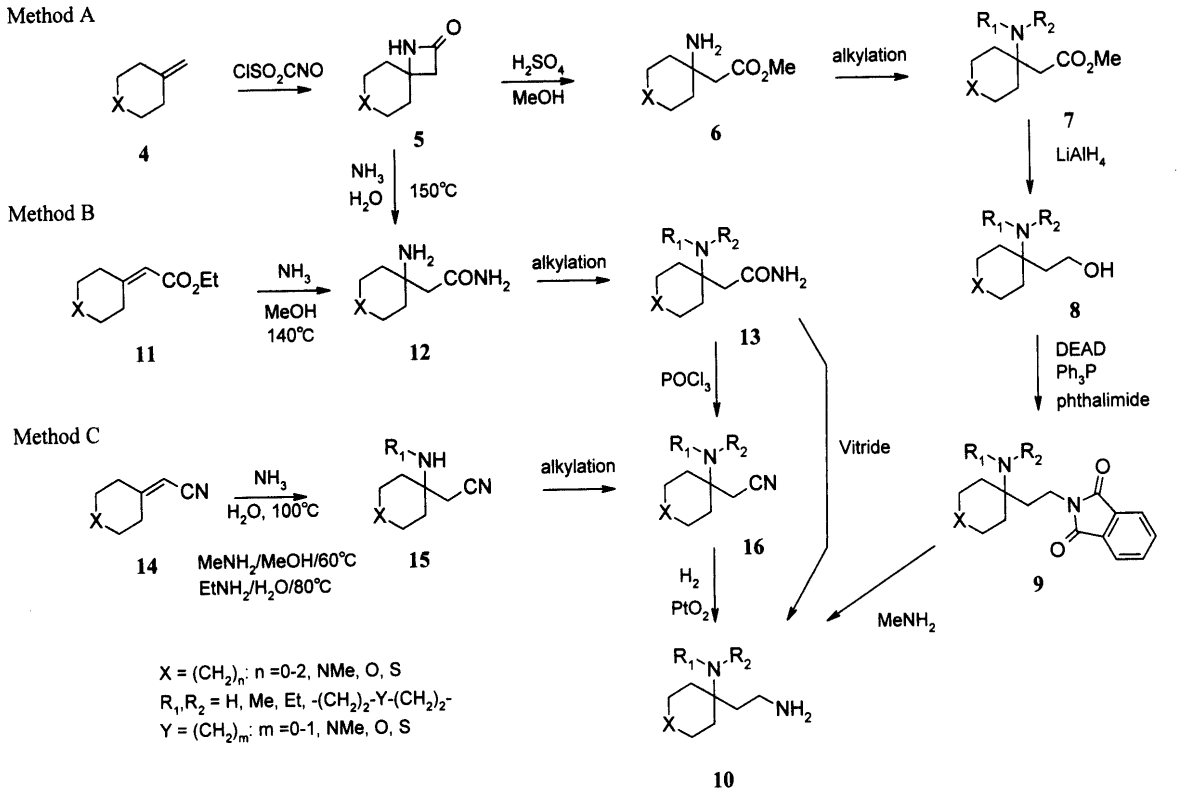


2 3 1

Chart 2



4 5 6 7 8 9 10 11 12 13 14 15 16

Chart 3

based on 5-HT_4 agonism. Cisapride,[14] which is already being used clinically, and SC-53116[15] also have the same parent skeleton. These compounds have cyclic amines and possess much more potent 5-HT_4 agonism than metoclopramide.[9] These improvements in 5HT_4 agonism are ascribed to cyclization of the amines, which serves to restrict free rotation around the amines, the direction of the lone pair on the basic nitrogen, and the relative location between the amine and the central part, and further, to increase basicity of the amines.

From the above reasoning, we planned to restrict the conformation of the amine by an indirect method using steric repulsion of neighboring substituents and synthesized a series of 4-amino-*N*-[2-(1-aminocycloalkan-1-yl)ethyl]-5-chloro-2-methoxybenzamide derivatives (**1**) which possessed geminal cyclic rings at the α-position of the basic nitrogen atom.

In this paper, we report the synthesis of the series **1**, and discuss structure–activity relationships (SAR) for 5-HT_4 agonism on the longitudinal muscle myenteric plexus (LMMP), and gastroprokinetic activities and the stereochemistry of **1c**.

Chemistry A series of 4-amino-*N*-[2-(1-aminocycloalkan-1-yl)ethyl]-5-chloro-2-methoxybenzamides (**1**) were prepared from 1-benzotriazolyl ester (activated ester) (**3**) by condensation with amines (**10**) in *N*,*N*-dimethylformamide (DMF) or dichloromethane (CH_2Cl_2) in good yield (Chart 2). Activated ester (**3**) was obtained from the benzoic acid by treatment with 1-hydroxybenzotriazole (HOBT) in the presence of dicyclohexylcarbodiimide (DCC) in DMF for more than one day in the absence of amines. Compound **3** is stable enough to be kept at room temperature for a long time without self-condensation, and is a new and effective intermediate for preparation of **1**.

2-(1-Aminocycloalkan-1-yl)ethylamine derivatives (**10**), key intermediates for **1**, were synthesized by the three methods (method A, B, C) shown in Chart 3.

Method A: β-Lactams (**5**), prepared according to the method of Durst and O'Sullivan,[16] were treated with sulfuric acid-methanol to give aminoesters (**6**), which were then alkylated with alkylhalide and reduced with lithium aluminium hydride to afford *N*-alkylated aminoalcohols (**8**). Introductions of an amine moiety into **8** proceeded by displacement with phthalimide and the resulting **9** were converted into the desired diamines (**10**) by deprotection.

Method B: Cycloalkylideneacetates (**11**) were treated with ammonia at 140 °C to give aminoacetamides (**12**) in

variable yield accompanied by cycloalkylideneacetamides by-products.[17] The *N*-alkylations of **12** were performed with alkylhalides, since reductive *N*-alkylation of **12** by treatment with formaldehyde and sodium triacetoxyborohydride gave hexahydro-1,3-pyrimidin-4-one derivatives preferentially.[17] Compounds **13** were reduced to afford the desired diamines (**10**).

Method C: Cycloalkylideneacetonitriles (**14**) were treated with amines (ammonia, methylamine, ethylamine) at 60—100 °C to afford **15** in good yield. These reaction temperatures were about 50 °C lower than those required to produce esters (**11**). Aminonitriles (**15**) were *N*-alkylated and hydrogenated over platinum oxide to afford dihydrochloride salts of the desired diamines in good yields.

In the case of diethylamine (**8i**), the second ethyl substituent was introduced by acetylation and reduction due to poor reactivity of methyl 2-(1-ethylamino-1-cyclohexyl)acetate with iodoethane due to steric hindrance. β-Lactams (**5**) were converted into **12** by treatment with ammonia and **13** were converted into **16** by reaction with phosphorous chloride. Method C was superior to the other two methods in terms of yields and ease of post-treatment to obtain the diamines (**10**).

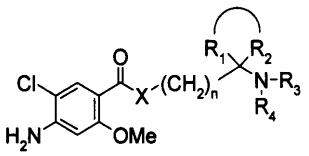
Results and Discussion

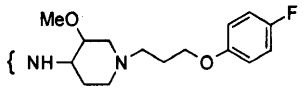
The 5-HT_4 agonistic effects of **1** were determined using LMMP of guinea pig ileum.[18-20] Results were expressed as EC_{50} values and the percentage of maximal responses for 5-HT_4 receptor-mediated contractions, which were calculated by subtracting the twitch responses obtained under 5-methoxytryptamine (5-MOT; 5-HT_4 agonist)-desensitized conditions from those obtained with the test compound alone.

The effect of length of the linker group between amine and amide and the introduction of geminal cyclic systems within 4-amino-*N*-(ω-aminoalkyl)-5-chloro-2-methoxybenzamides are indicated by compounds **1a**—**1e**. Metoclopramide and dimethylaminopropyl derivative (**1a**) showed weak agonistic activities on LMMP contraction. In ω-aminoalkyl compounds having a geminal cyclohexane at the α position of the basic amine, **1c** possessing an ethylene linker ($n=2$) had potent 5-HT_4 agonistic activity, whereas **1b**[21] ($n=1$) and **1d** ($n=3$) only had weak activities. Compound **1e**, which is a ring-opened derivative of **1c** showed moderate activity. Thus, the introduction of a geminal cyclohexane ring brought about a pronounced improvement of 5-HT_4 agonistic activity (**1c** *versus* **1a**).

Subsequent modifications of the side chain were performed on the basis of the structure of **1c**. The effects of *N*-substituents and cyclization of basic amines are shown by compounds **1f**—**1n**. Non-substituted amine (**1f**) and methylamine (**1g**) had slightly reduced activity compared with dimethylamine (**1c**), ethylmethylamine (**1h**), and diethylamine (**1i**); oveall, activity was gradually

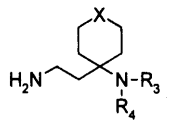
Table 1. Enhancing Effect of Test Compounds on the Twitch Response in Guinea Pig Ileum



Compd. No.	X	*n*	R_1	R_2	R_3	R_4	5-HT_4 agonism[a] EC_{50} (μM)	Maximal response[a] (%)
1a	NH	2	H	H	CH_3	CH_3	5.0	34
1b	NH	1	$(CH_2)_5$		CH_3	CH_3	7.0	34
1c	NH	2	$(CH_2)_5$		CH_3	CH_3	1.0	146
1d	NH	3	$(CH_2)_5$		CH_3	CH_3	—	30>
1e	NH	2	C_2H_5	C_2H_5	CH_3	CH_3	4.2	72
1f	NH	2	$(CH_2)_5$		H	H	2.5	120
1g	NH	2	$(CH_2)_5$		H	CH_3	2.2	129
1h	NH	2	$(CH_2)_5$		CH_3	C_2H_5	0.54	104
1i	NH	2	$(CH_2)_5$		C_2H_5	C_2H_5	0.67	63
1j	NH	2	$(CH_2)_5$		$(CH_2)_4$		0.44	132
1k	NH	2	$(CH_2)_5$		$(CH_2)_5$		0.80	87
1l	NH	2	$(CH_2)_5$		$(CH_2)_2O(CH_2)_2$		1.2	60
1m	NH	2	$(CH_2)_5$		$(CH_2)_2S(CH_2)_2$		2.3	33
1n	NH	2	$(CH_2)_5$		$(CH_2)_2NMe(CH_2)_2$		—	30>
1o	NH	2	$(CH_2)_4$		CH_3	CH_3	1.6	123
1p	NH	2	$(CH_2)_6$		CH_3	CH_3	5.2	72
1q	NH	2	$(CH_2)_2O(CH_2)_2$		CH_3	CH_3	3.7	93
1r	NH	2	$(CH_2)_2S(CH_2)_2$		CH_3	CH_3	1.3	111
1s	NH	2	$(CH_2)_2NMe(CH_2)_2$		CH_3	CH_3	3.8	63
17	O	2	$(CH_2)_5$		CH_3	CH_3	5.7	54
Metoclopramide	NH	1	H	H	C_2H_5	C_2H_5	3.2	49
Cisapride							0.15	108

a) The EC_{50} values and percentages of maximal responses for 5-HT_4 receptor-mediated contractions were calculated by subtracting the twitch responses on LMMP obtained under the 5-MOT (5-HT_4 agonist)-desensitized condition, from these obtained with the test compound alone.

Table 2. Spectral Data for 2-(1-Aminocycloalkan-1-yl)ethylamine Derivatives



Compd. No.	X	R_3	R_4	Method	MS m/z $M^{+a)}$	^{1}H-NMR ($CDCl_3$) δ (ppm)
10c	CH_2	CH_3	CH_3	A, B, C	166	1.30—1.80 (10H, m), 2.34 (6H, s), 2.41 (2H, br s)
10f	CH_2	H	H	C	148	1.20—1.90 (12H, m), 2.69—2.75 (2H, m)
10g	CH_2	CH_3	H	C	152	1.20—1.85 (15H, m), 2.29 (3H, s), 2.55—2.75 (2H, m)
10h	CH_2	CH_3	C_2H_5	C	180	1.07 (3H, t, J=7 Hz), 1.20—1.85 (12H, m), 2.23 (3H, s), 3.80 (2H, q, J=7 Hz)
10i	CH_2	C_2H_5	C_2H_5	A	198	1.01 (6H, t, J=7 Hz), 1.15—1.75 (14H, m), 2.57 (4H, q, J=7 Hz), 2.69 (2H, t, J=8 Hz).
10j	CH_2	$(CH_2)_4$		A	197 (M^++1)	0.90—1.90 (16H, m), 2.45—2.90 (6H, m)
10k	CH_2	$(CH_2)_5$		B	210	1.00—1.90 (18H, m), 2.30—2.80 (6H, m)
10l	CH_2	$(CH_2)_2O(CH_2)_2$		B	213 (M^++1)	0.90—2.20 (12H, m), 2.40—3.00 (6H, m), 3.50—3.90 (4H, m)
10m	CH_2	$(CH_2)_2S(CH_2)_2$		B	228	1.20—1.90 (12H, m), 2.50—3.00 (10H, m)
10n	CH_2	$(CH_2)_2NMe(CH_2)_2$		B	226	1.10—1.80 (12H, m), 2.25 (3H, s), 2.25—2.75 (10H, m)
10o	—	CH_3	CH_3	A	157 (M^++1)	1.40—1.45 (2H, m), 1.55—1.67 (6H, m), 1.73—1.78 (2H, m), 2.23 (6H, s), 2.78 (2H, t, J=8 Hz)
10p	$(CH_2)_2$	CH_3	CH_3	A	185 (M^++1)	1.40—1.80 (14H, m), 2.22 (6H, s), 2.67—2.85 (2H, m)
10q	O	CH_3	CH_3	A	173 (M^++1)	1.30—1.75 (8H, m), 2.24 (6H, s), 2.62—2.83 (2H, m), 3.48—3.96 (4H, m)
10r	S	CH_3	CH_3	C	188	1.35—2.00 (6H, m), 2.19 (6H, s), 2.60—3.15 (4H, m), 3.60—3.78 (2H, m)
10s	NMe	CH_3	CH_3	A	185	1.30—1.90 (8H, m), 2.22 (6H, s), 2.27 (3H, s), 2.34—2.80 (4H, m)

a) Compounds **10j**, **10l**, **10o**, **10p**, and **10q** were measured by FAB-MS.

reduced with increasing size of the *N*-substituents. In cyclic amines, pyrrolidine (**1j**) showed more potent activity than **1c**, but piperidine (**1k**) showed less activity than **1c**. Thus, relatively small sized dialkylated amine derivatives (**1c**, **1j**) tended to show potent activities in comparison with expanded amine derivatives (**1j**, **1k**). Morpholine (**1l**) and thiomorpholine (**1m**) showed weaker activities than piperidine (**1k**). In this case, a decrease of basicity of the amine also seemed to affect the potencies, since the piperazine (**1n**) almost lost the activity.

The effects of geminal cyclic rings at the α position of the basic amine are shown by compounds **1o**—**1s**. Initially, the effects of ring size for cyclic systems were evaluated. Similar to cyclohexane (**1c**), cyclopentane (**1o**) retained activity, but cycloheptane (**1p**) showed greatly reduced activity. Replacement of carbon atom by heteroatoms at 4-position of the cyclohexane ring of **1c** also reduced the activities, in the order of their polarity (**1r** > **1q** > **1s**).

From biological results for **1c** and its derivatives, the geminal cyclic ring play an important role in potency as a 5-HT_4 agonist. That is, this part presumably functions as a lipophilic interaction site with the 5-HT_4 receptor and restricts the conformation of the side chain. As a result, it may resemble the circumstances around the amine group like cyclic amines.

The preferred distance between the amide and amine was a three-carbon chain length which is a little longer than with potent 5-HT_3 antagonists, as shown in our previous investigation of 5-HT_3 antagonists[22,23] and the SAR of SC-53116.[15]

Replacement of the amide bond by an ester bond reduced 5-HT_4 agonism in **17**. It is well known that 4-amino-5-chloro-2-methoxybenzamides have intramolecular hydrogen bonding between the hydrogen of benzamide and the oxygen of 2-methoxy substituent, and form a pseudo 6-membered ring. However, **17** does not have intramolecular hydrogen bonding so that it can rotate freely at the ester bond. This conformational difference presumably influences the 5-HT_4 agonistic potencies of **1c** and **17**. Recently, some ester derivatives were reported as 5-HT_4 antagonists,[24-26] however, **17** only had weak 5-HT_4 antagonistic activity

We selected the potent 4-amino-5-chloro-*N*-[2-(1-dimethylamino-1-cyclohexyl)ethyl]-2-methoxybenzamide (**1c**, YM-47813) for *in vitro* test, and also evaluated it for *in vivo* effect on gastric motility[27] and gastric emptying.[28] The effects of YM-47813 and cisapride on antral motility in the digestive state in conscious dogs by oral administration are shown in Fig. 1. Gastric antral motility was quantified using the motility index (MI) every 15 min period, for 180 min after administration of test compound, and is expressed as a percentage to the mean MI (basal MI) of 4 sequential 15 min periods before administration. The effects of YM-47813 and cisapride on gastric emptying in a canine gastroparesis model by oral administration are shown in Fig. 2. Gastric emptying activity was evaluated as the recovery by test compounds from the delay of gastric emptying caused by s.c. injection of UK14,304 (adrenaline α_2 agonist) just before feeding and is expressed as a concentration of plasma acetoaminophen by the dye method. Consequently, YM-47813 effectively enhanced gastric motility and gastric emptying by oral adiministration (1—3 mg/kg) in compraison with cisapride, and its potent gastroprokinetic activities were confirmed.

Table 3. Spectral Data for 4-Amino-*N*-[2-(1-aminocycloalkan-1-yl)ethyl]-5-chloro-2-methoxybenzamides

Compd. No.	Yield (%)[a]	mp (°C)[b]	MS *m/z* M^{+c}	^{1}H-NMR ($CDCl_3$) δ (ppm)	Formula	Elemental analysis (%) Calcd (Found)			
						C	H	Cl	N
1c	86	161—163	354, 356 ($M^+ + 1$)	1.33—1.50 (6H, m), 1.53—1.68 (4H, m), 1.71 (2H, t, J=9Hz), 2.26 (6H, s), 3.48—3.50 (2H, m), 3.89 (3H, s), 4.42 (2H, br s), 6.30 (1H, s), 7.85 (1H, br s), 8.11 (1H, s)	$C_{18}H_{28}ClN_3O_2$	61.09 (60.89)	7.97 (8.09)	10.02 (10.11)	11.87 (11.74)
1d	55	163—165	367, 369	1.24—1.63 (14H, m), 2.33 (6H, s), 3.22—3.24 (2H, m), 3.82 (3H, s), 5.91 (2H, s), 6.48 (1H, s), 6.51 (1H, s), 7.67 (1H, s), 7.92 (1H, s)/(DMSO-d_6)	$C_{19}H_{30}ClN_3O_2$ $0.5C_4H_4O_4$ H_2O	56.81 (56.77)	7.72 (7.58)	7.99 (8.05)	9.47 (9.30)
1e	16	124—125	342, 344 ($M^+ + 1$)	0.93 (6H, t, J=8 Hz), 1.50—1.66 (4H, m), 1.74 (2H, t, J=8 Hz), 2.41 (6H, s), 3.44—3.49 (2H, m), 3.90 (3H, s), 4.39 (2H, s), 6.30 (1H, s), 7.85 (1H, br s), 8.10 (1H, s)	$C_{17}H_{28}ClN_3O_2$ $0.3H_2O$	58.80 (58.96)	8.30 (8.23)		12.10 (11.99)
1f	40	103—105	325, 327	1.05—1.80 (14H, m), 3.35—3.70 (2H, m), 3.86 (3H, s), 4.37 (2H, br s), 6.28 (1H, s), 8.09 (1H, br), 8.10 (1H, s)	$C_{16}H_{24}ClN_3O_2$	59.98 (59.80)	7.42 (7.55)	10.88 (11.01)	12.90 (12.69)
1g	73	115—117	339, 341	1.05—1.80 (12H, m), 2.28 (3H, s), 3.30—3.60 (2H, m), 3.87 (3H, s), 4.39 (2H, br s), 6.29 (1H, s), 8.08 (1H, br), 8.10 (1H, s)	$C_{17}H_{26}ClN_3O_2$	60.08 (60.06)	7.71 (7.76)	10.43 (10.32)	12.36 (12.33)
1h	58	144—145	367, 369	1.00—2.70 (18H, m), 3.46 (2H, br s), 3.90 (3H, s), 4.38 (2H, s), 6.30 (1H, s), 7.82 (1H, br s)	$C_{19}H_{30}ClN_3O_2$	62.03 (61.83)	8.22 8.35	9.64 9.87	11.42 (11.20)
1i	57	117—122	381, 383	1.03 (6H, br s), 1.26—1.80 (12H, m), 2.61 (4H, br s), 3.42 (2H, br s), 3.89 (3H, s), 4.35 (2H, s), 6.29 (1H, s), 7.67 (1H, br s), 8.09 (1H, s)	$C_{20}H_{32}ClN_3O_2$ $0.5H_2O$	61.44 (61.49)	8.51 (8.27)		10.75 (10.79)
1j	38	138—141	380, 382 ($M^+ + 1$)	1.25—1.90 (16H, m), 2.60—2.90 (4H, m), 3.40—3.55 (2H, m), 3.90 (3H, s), 4.35 (2H, s), 6.29 (1H, s), 7.85 (1H, br s), 8.08 (1H, s)	$C_{20}H_{30}ClN_3O_2$	63.23 (62.91)	7.96 (7.96)	9.33 (9.33)	11.06 (11.06)
1k	54	149—150	394, 396 ($M^+ + 1$)	1.30—1.52 (12H, m), 1.60—1.75 (6H, m), 2.51 (4H, br s), 3.38—3.43 (2H, m), 3.89 (3H, s), 4.39 (2H, s), 6.29 (1H, s), 7.65 (1H, br s), 8.09 (1H, s)	$C_{21}H_{32}ClN_3O_2$ $0.1H_2O$	63.73 (63.54)	8.20 (8.21)	8.96 (9.16)	10.62 (10.59)
1l	89	184—186	396, 398 ($M^+ + 1$)	1.37—1.41 (6H, m), 1.60—1.70 (6H, m), 2.57—2.60 (4H, m), 3.39—3.44 (2H, m), 3.66—3.69 (4H, m), 3.90 (3H, s), 4.35 (2H, s), 6.29 (1H, s), 7.63 (1H, br s), 8.11 (1H, s)	$C_{20}H_{30}ClN_3O_3$ $0.2H_2O$	60.13 (60.01)	7.75 (7.66)		10.52 (10.42)
1m	41	165—167	412, 414 ($M^+ + 1$)	1.26—1.38 (5H, m), 1.52—1.74 (7H, m), 2.63—2.65 (4H, m), 2.89 (4H, br s), 3.36—3.41 (2H, m), 3.90 (3H, s), 4.38 (2H, s), 6.29 (1H, s), 7.60 (1H, br s), 8.11 (1H, s)	$C_{20}H_{30}ClN_3O_2S$ $0.2H_2O$	57.75 (57.72)	7.37 (7.12)	8.53 (8.66)	10.11 (10.02)
1n	40	183—185	409, 411 ($M^+ + 1$)	1.25—1.45 (6H, m), 1.55—1.85 (6H, m), 2.28 (3H, s), 2.44 (4H, br s), 2.64 (4H, br s), 3.38—3.43 (2H, m), 3.89 (3H, s), 4.35 (2H, s), 6.29 (1H, s), 7.63 (1H, br s), 8.10 (1H, s)	$C_{21}H_{33}ClN_4O_2$	61.67 (61.51)	8.13 (8.25)	8.67 (8.55)	13.70 (13.53)
1o	68	185—187	339, 341	1.46—1.50 (2H, m), 1.56—1.67 (4H, m), 1.78—1.83 (4H, m), 2.27 (6H, s), 3.49—3.53 (2H, m), 3.89 (3H, s), 4.35 (2H, br s), 6.29 (1H, s), 7.93 (1H, br s), 8.12 (1H, s)	$C_{17}H_{26}ClN_3O_2$ $0.4H_2O$	58.83 (58.66)	7.78 (7.59)		12.11 (12.07)
1p	74	137—138	367, 369	1.35—1.80 (14H, m), 2.20 (6H, s), 3.28 (2H, s), 3.82 (3H, s), 5.89 (2H, s), 6.47 (1H, s), 7.67 (1H, s), 7.98 (1H, m)	$C_{19}H_{30}ClN_3O_2$ $0.75H_2O$	59.83 (59.91)	8.32 (8.21)		11.02 (11.34)
1q	51	216—219	356, 358 ($M^+ + 1$)	1.50—1.56 (2H, m), 1.68—1.78 (4H, m), 2.32 (6H, s), 3.26—3.31 (2H, m), 3.43—3.52 (2H, m), 3.70—3.75 (2H, m), 3.83 (3H, s), 5.92 (2H, s), 6.47 (1H, s), 6.57 (1H, s), 7.70 (1H, s), 8.00 (1H, br s)/(DMSO-d_6)	$C_{17}H_{26}ClN_3O_3$ $0.5C_4H_4O_4$ $0.6H_2O$	53.73 (53.94)	6.93 (6.66)	8.34 (8.06)	9.89 (9.51)
1r	53	162—163	372, 374 ($M^+ + 1$)	1.64—1.73 (2H, m), 1.73—1.81 (2H, m), 2.00—2.10 (2H, m), 2.23 (6H, s), 2.40—2.50 (2H, m), 2.88—2.98 (2H, m), 3.36—3.44 (2H, m), 3.90 (3H, s), 4.39 (2H, s), 6.30 (1H, s), 7.74 (1H, br s), 8.11 (1H, s)	$C_{17}H_{26}ClN_3O_2S$ $0.3H_2O$	54.11 (54.38)	7.11 (6.85)	9.40 (9.45)	11.14 (11.12)
1s	40	217—218	368, 370	1.50—1.70 (4H, m), 1.74—1.84 (2H, m), 2.20 (6H, s), 2.36 (3H, s), 2.52—2.73 (4H, m), 3.18—3.30 (2H, m), 3.82 (3H, s), 5.90 (2H, s), 6.46 (1H, s), 6.47 (1H, s), 7.69 (1H, s), 7.96 (1H, br s)/(DMSO-d_6)	$C_{18}H_{29}ClN_4O_2$ $0.5C_4H_4O_4$ $0.5H_2O$	55.10 (55.40)	7.40 (7.23)	8.13 (8.15)	12.85 (12.71)
17	68	137—138	354, 356	1.32—1.50 (6H, m), 1.54—1.72 (4H, m), 1.86 (2H, t, J=7Hz), 2.27 (6H, s), 3.85 (3H, s), 4.32 (2H, t, J=7Hz), 4.44 (2H, s), 6.29 (1H, s), 7.83 (1H, s)	$C_{18}H_{27}ClN_2O_3$	60.92 (60.70)	7.67 (7.70)		7.89 (7.85)

a) The yield from the corresponding diamines **10** after crystallization. *b*) Acid free compounds and fumarates were crystallized from AcOEt–hexane and EtOH–AcOEt respectively. *c*) Compounds **1c**, **1e**, **1j**, **1k**, **1l**, **1m**, **1n**, **1q**, and **1r** were measured by FAB-MS.

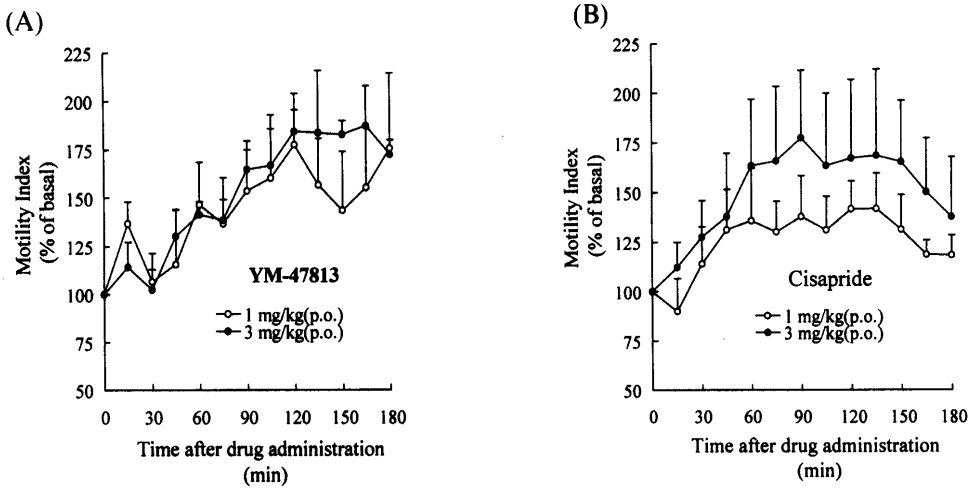


Fig. 1. The Effects of Orally Administered YM-47813 (A) and Cisapride (B) on Antral Motility in the Digestive State in Conscious Dogs

Gastric antral motility was quantified by using MI of each 15 min period for 180 min after administration of test compound, and is expressed as percentage of the mean MI (basal MI) of 4 sequential 15 min periods for 60 min before administration. Each point represents the mean ± S.E.M. for 3 to 4 dogs.

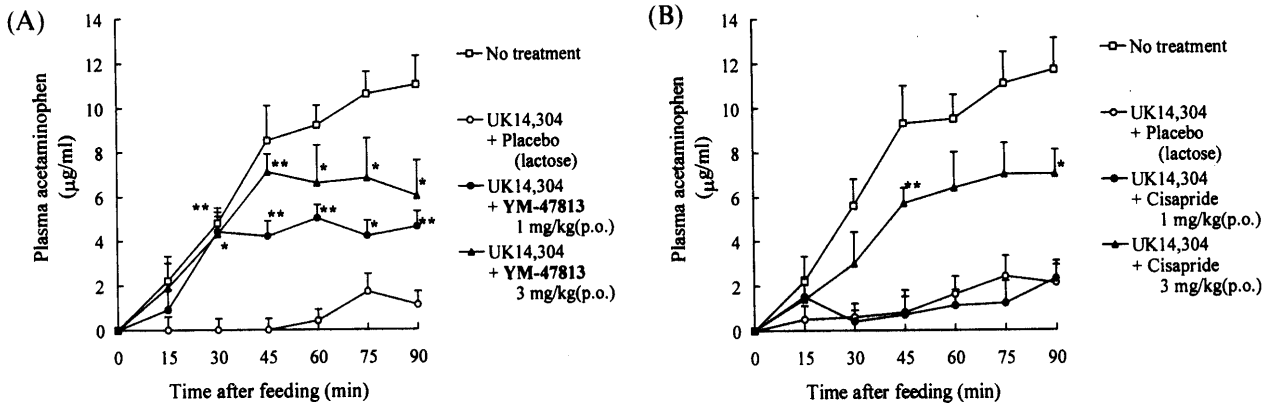


Fig. 2. The Effects of YM-47813 (A) and Cisapride (B) on Gastric Emptying in the Canine Gastroparesis Model

Test compounds were orally administered 30 min before feeding. Delay of gastric emptying was induced by s.c. injection of UK14,304 (adrenaline α_2 agonist, 0.02 mg/kg) just before feeding, and a mixture of canned food (20 g/kg) and acetaminophen (50 mg/kg) was then given to each dog. Blood samples were obtained at 0, 15, 30, 45, 60, 75 and 90 min after feeding, and the plasma acetaminophen concentration was measured by the dye method with a spectrophotometer. Each point represents the mean ± S.E.M. for 6 dogs. $*p<0.05$; $**p<0.01$ significant differences from the vehicle treatment.

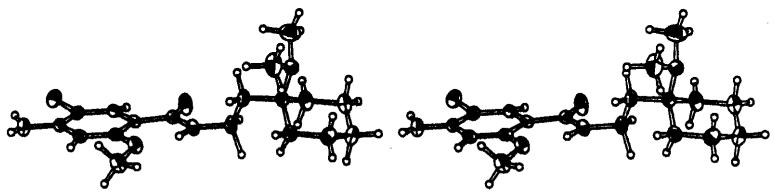


Fig. 3. Stereoview of the Crystal Structure of YM-47813

Results of the X-ray analysis of YM-47813 are shown in Fig. 3. YM-47813 adopted a pseudo 6-membered ring by intramolecular hydrogen bonding and had an axial dimethylamino substituent on the cyclohexane ring. Since there are two chair conformational isomers involving the dimethylamino substitutent in the cyclohexane ring of YM-47813 (axial and equatorial conformations) are possible, we calculated the energy differences between them using the MOPAC package of AM1 and PM3 calculations, fixing both conformations to overlap the parts of the molecules without the cyclohexane ring, on the basis of X-ray data. As a result, only a 3—4 kcal/mol energy difference between them was predicted (final heat of formation (AM1); −68.72396 kcal/mol (axial), −72.86003 kcal/mol (equatorial), $\Delta E = 4.13607$ kcal/mol, final heat of formation (PM3); −80.71086 kcal/mol (axial), −83.44732 kcal/mol (equatorial), $\Delta E = 2.73646$ kcal/mol.). Thus, these isomers can easily convert into each other. When considering the location of the lipophilic part in the cyclic amine, and the potency of YM-47813 and **1j**, the axial isomer is more likely to be the active conformation for a 5-HT_4 agonist than the equatorial isomer.

In conclusion, we synthesized a novel series of 4-amino-*N*-[2-(1-aminocycloalkan-1-yl)ethyl]-5-chloro-2-methoxybenzamides (**1**) and evaluated their 5-HT_4 agonistic activities on LMMP in guinea pig ileum. Among them,

YM-47813 showed potent 5-HT_4 agonistic activity in *in vitro* tests and effective gastroprokinetic activities in *in vivo* tests and was selected as a promising candidate for a new gastroprokinetic agent.

Experimental

All melting points were determined on a Yanaco MP-500D melting point apparatus and are uncorrected. ^{1}H-NMR spectra were measured with a JEOL FX90Q, a FX100, a FX270 or FX400 spectrometer; chemical shifts are reported in δ units using tetramethylsilane as internal standard and the following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, dt = double triplet, br = broad. Mass spectra were recorded with a Hitachi M-80 electron impact (EI), JEOL JMS-DX300 (FAB) spectrometer or Hewlett Packard 5970 MSD (GC) spectrometer. Elemental analyses were performed with a Yanaco MT-5. All organic extracts were dried over anhydrous magnesium sulfate and concentrated with a rotary evaporator under reduced pressure.

1-Azaspiro[3,5]nonan-2-one (5) Chlorosulfonyl isocyanate (7.30 g, 51.5 mmol) was added dropwise to a solution of methylenecyclohexane (**4**) (4.80 g, 50 mmol) in ether (50 ml) at 0 °C and the mixture was stirred at room temperature for 30 min. A suspension of sodium thiosulfate (20 g, 126 mmol) in H_2O and 10% aqueous KOH were simultaneously added to the reaction mixture, keeping temperature under 5 °C, and at pH about 10, and the mixture stirred for 2 h. The reaction mixture was then extracted with ether. The extract was washed with brine, dried, and concentrated to afford **5** as a colorless oil (6.04 g, 87%). ^{1}H-NMR ($CDCl_3$) δ: 1.20—1.90 (10H, m), 2.62 (2H, d, J = 2 Hz), 6.70 (1H, br). GC-MS m/z: 139 (M^+).

Methyl 2-(1-Amino-1-cyclohexyl)acetate (6f) A solution of **5** (6.76 g, 48.7 mmol) and conc. H_2SO_4 (5 ml) in MeOH (150 ml) was refluxed overnight. The reaction mixture was concentrated and the residue was diluted with AcOEt and extracted with dil. HCl. The extract was basified with K_2CO_3 and extracted with AcOEt. The extract was washed with brine, dried, and concentrated. The residue was chromatographed on Al_2O_3 eluting with $CHCl_3$–MeOH (10 : 1) to afford **6f** as a colorless oil (7.65 g, 92%). ^{1}H-NMR ($CDCl_3$) δ: 1.20—1.75 (10H, m), 1.62 (2H, s), 2.40 (2H, s), 3.68 (3H, s). GC-MS m/z: 171 (M^+).

Methyl 2-(1-Dimethylamino-1-cyclohexyl)acetate (7c) A mixture of **6f** (1.71 g, 10 mmol), 35% formaldehyde solution (20 ml), and formic acid (20 ml) was stirred at 110 °C for 2 h. The reaction mixture was concentrated and the residue basified with aqueous K_2CO_3 and extracted with AcOEt. The extract was washed with brine, dried, and concentrated to afford **7c** as a colorless oil (1.86 g, 93%). ^{1}H-NMR ($CDCl_3$) δ: 1.20—1.95 (10H, m), 2.22 (6H, s), 2.32 (2H, s), 3.65 (3H, s). EI-MS m/z: 199 (M^+).

2-(1-Dimethylamino-1-cyclohexyl)ethanol (8c) To a suspension of lithium aluminium hydride (0.20 g, 5.3 mmol) in tetrahydrofuran (THF, 20 ml) was added **7c** (1.00 g, 5.0 mmol) at 0 °C and the mixture was stirred for 30 min. Sodium sulfate decahydrate (3 g) was added to the reaction mixture at 0 °C and the mixture was filtered after stirring for 2 h. The filtrate was concentrated and chromatographed on Al_2O_3 eluting with $CHCl_3$–MeOH (10 : 1) to afford **8c** as a colorless oil (0.89 g, quant.). ^{1}H-NMR ($CDCl_3$) δ: 1.10—1.90 (10H, m), 1.80 (2H, t, J = 6 Hz), 2.27 (6H, s), 3.80 (2H, t, J = 6 Hz), 6.70 (1H, br). EI-MS m/z: 171 (M^+).

***N*-[2-(1-Dimethylamino-1-cyclohexyl)ethyl]phthalimide (9c)** Diethyl azodicarboxylate (DEAD, 1.04 g, 6.0 mmol) was added dropwise to a solution of **8c** (0.84 g, 4.9 mmol), triphenylphosphine (1.96 g, 7.5 mmol) and phthalimide (0.96 g, 6.5 mmol) in THF (15 ml) at 5 °C over 2 min and stirred for 30 min. The reaction mixture was concentrated and the residue diluted with AcOEt and extracted with dil. HCl. The extract was basified with K_2CO_3 and extracted with AcOEt. The extract was washed with brine, dried, and concentrated. The residue was chromatographed on SiO_2 eluting with $CHCl_3$–MeOH (10 : 1) to afford **9c** as a colorless oil (0.41 g, 28%). ^{1}H-NMR ($CDCl_3$) δ: 1.15—2.00 (12H, m), 2.29 (6H, s), 3.60—3.85 (2H, m), 7.60—7.95 (4H, m). EI-MS m/z: 300 (M^+)

2-(1-Amino-1-cyclohexyl)acetamide (12f) Preparation from **5**: A solution of **5** (39.0 g, 280 mol) in 29% aq. ammonia was heated at 150 °C in an autoclave for 15 h. The reaction mixture was concentrated and crystallized from AcOEt–hexane to afford **12f** as a white solid (39.0 g, 89%). Preparation from **11**: A solution of ethyl cyclohexylideneacetate (**11**, 0.84 g, 5.0 mmol) in saturated ammonia–methanol (30 ml) was heated at 140 °C in a sealed tube for 3 d. The reaction mixture was concentrated, diluted with AcOEt and extracted with dil. HCl. The extract was basified with K_2CO_3 and concentrated. Methanol was added to the residue and precipitates were removed by filtration. The filtrate was concentrated and the residue was chromatographed on SiO_2 eluting with $CHCl_3$–MeOH–aq. ammonia (10 : 1.5 : 0.2) to afford **12f** (0.57 g, 74%). ^{1}H-NMR ($CDCl_3$) δ: 1.30—1.70 (10H, m), 1.72 (2H, s), 2.26 (2H, s), 6.35 (1H, br), 7.92 (1H, br). EI-MS m/z: 156 (M^+).

2-(1-Dimethylamino-1-cyclohexyl)acetamide (13c) Iodomethane (55.0 g, 388 mmol) was added dropwise to a stirred suspension of **12f** (30.3 g, 194 mmol) and powdered K_2CO_3 (53.5 g, 388 mmol) in CH_3CN (500 ml) maintained at 10 °C and stirred for 5 h. The reaction mixture was filtered and concentrated. The residue was diluted with water and extracted with AcOEt. The extract was washed with brine, dried, and concentrated to afford crude **13c** (29.5 g). The residue was crystallized from AcOEt–hexane to afford **13c** as a white solid (18.8 g, 54%). mp 252—255 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.30—1.80 (10H, m), 2.32 (6H, s), 2.53 (2H, s). EI-MS m/z: 184 (M^+).

2-(1-Amino-1-cyclohexyl)acetonitrile (15f) A solution of **14** (0.36 g, 3.0 mmol) in 29% aqueous ammonia (15 ml) and methanol (5 ml) was heated at 100 °C in a sealed tube for 20 h. The reaction mixture was concentrated, and the residue was chromatographed on SiO_2 eluting with $CHCl_3$–MeOH (10 : 1) to afford **15f** as a colorless oil (0.35 g, 85%). ^{1}H-NMR ($CDCl_3$) δ: 1.30—1.80 (10H, m), 2.16 (2H, br s), 2.49 (2H, s). HR-MS (EI) Calcd for $C_8H_{14}N_2$ (M^+) 138.1157, Found 138.1161.

2-(1-Methylamino-1-cyclohexyl)acetonitrile (15g) A solution of **14** (10.94 g, 90.3 mmol) in 40% methylamine–methanol (55 ml) was heated at 60 °C in a sealed tube for 18 h. The reaction mixture was concentrated and the residue was diluted with AcOEt and extracted with dil. HCl. The extract was basified with K_2CO_3 and extracted with AcOEt. The extract was washed with brine, dried, and concentrated to afford **15g** as a colorless oil (12.20 g, 89%). ^{1}H-NMR ($CDCl_3$) δ: 1.00—1.90 (10H, m), 2.31 (3H, s), 2.50 (2H, s). HR-MS (EI) Calcd for $C_9H_{16}N_2$ (M^+) 152.1313, Found 152.1314.

2-(1-Ethylamino-1-cyclohexyl)acetonitrile (15i) A solution of **14** (0.36 g, 3.0 mmol) in 70% ethylamine–H_2O (5 ml) was heated at 80 °C in a sealed tube for 20 h. The reaction mixture was concentrated and the residue was diluted with AcOEt and extracted with dil. HCl. The extract was basified with K_2CO_3 and extracted with AcOEt. The extract was washed with brine, dried, and concentrated. The residue was chromatographed on SiO_2 eluting with AcOEt to afford **15i** as a colorless oil (0.40 g, 80%). ^{1}H-NMR ($CDCl_3$) δ: 1.12 (3H, t, J = 7 Hz), 1.20—1.85 (10H, m), 2.46 (2H, s), 2.53 (2H, q, J = 7 Hz). HR-MS (EI) Calcd for $C_{10}H_{18}N_2$ (M^+) 166.1470, Found 166.1473.

2-(1-Dimethylamino-1-cyclohexyl)acetonitrile (16c) Preparation from **13c**: Phosphorous oxychloride (5 ml) was added dropwise to a solution of **13c** (2.00 g, 10.9 mmol) in pyridine (50 ml) at 5 °C and the reaction mixture was stirred at 60 °C for 2 h. The reaction mixture was concentrated and the residue basified with aqueous K_2CO_3 and extracted with AcOEt. The extract was washed with brine, dried, and concentrated. The residue was chromatographed on SiO_2 eluting with $CHCl_3$–MeOH–aq. NH_3 (10 : 1 : 0.05) to afford **16c** as a yellow oil (1.56 g, 86%). Preparation from **15g**: Sodium cyanoborohydride (2.40 g, 63.4 mmol) was added portionwise to an emulsion of **15g** (8.92 g, 58.7 mmol), acetic acid (10 ml), and 30% formaldehyde solution (10 ml) in CH_2Cl_2 (100 ml) at 0 °C and the mixture was stirred at room temperature for 4 h. The mixture was basified with aqueous K_2CO_3 and extracted with $CHCl_3$–MeOH (10 : 1). The extract was washed with brine, dried, and concentrated to afford **16c** as a colorless oil (9.06 g, 93%). ^{1}H-NMR ($CDCl_3$) δ: 1.30—1.80 (10H, m), 2.34 (6H, s), 2.41 (2H, s). EI-MS m/z: 166 (M^+).

2-(1-Dimethylamino-1-cyclohexyl)ethylamine (10c) Preparation from **9c**: A solution of **9c** (0.40 g, 1.3 mmol) in 40% methylamine–methanol (10 ml) was stirred at 50 °C for 1 h. The reaction mixture was concentrated and the residue chromatographed on SiO_2 eluting with $CHCl_3$–MeOH–aq. NH_3 (10 : 2 : 0.2) to afford **10c** as a colorless oil (0.15 g, 66%). Preparation from **13c**: Vitride (70% sodium bis(2-methoxyethoxy)-aluminum solution in toluene, 32 ml) was added dropwise to a solution of **13c** (5.00 g, 27.1 mmol) in toluene (200 ml) and then refluxed for 3 h. The reaction mixture was cooled in an ice bath and treated with saturated $NaHCO_3$ solution. The precipitates were removed by filtration and washed with CH_3CN. The filtrate was concentrated and distilled to afford **10c** as a colorless oil (0.69 g, 15%). bp 58.5 °C (1.2 mmHg) Preparation from **16c**: A solution of **16c** (0.20 g, 1.2 mmol) in EtOH (10 ml) and 4 N HCl/AcOEt (0.6 ml) was treated with PtO_2 and hydrogenated at 40 °C

at 4 atmospheres for 15 h. The catalysts were removed by filtration and the filtrate was concentrated. The residue was crystallized from ether to afford **10c**·2HCl as a white solid (0.22 g, 89%). ^{1}H-NMR (DMSO-d_6) δ: 1.10—1.85 (10H, m), 2.20 (2H, m), 2.61 (6H, s), 3.00 (2H, m), 8.26 (3H, br), 10.55 (1H, br).

3-(1-Dimethylamino-1-cyclohexyl)propylamine (10d) A solution of 1-dimethylamino-1-cyclohexylcarboxaldehyde[29] (0.32 g, 2.1 mmol) in 1,2-dimethoxyethane (5 ml) was added to a suspension of diethyl cyanomethylphosphonate (0.44 g, 2.5 mmol) and 60% sodium hydride in oil (0.10 g, 2.5 mmol) in 1,2-dimethoxyethane (5 ml) at 0 °C and stirred at room temperature for 20 min. The reaction mixture was then concentrated and the residue diluted with water and extracted with AcOEt. The extract was washed with brine, dried, concentrated, and the resulting residue was chromatographed on SiO_2 eluting with AcOEt–hexane (1 : 1) to afford 3-(1-dimethylamino-1-cyclohexyl)acrylonitrile as a colorless oil (0.31 g, 84%). A solution of this nitrile (0.48 g, 2.7 mmol) in EtOH (10 ml) and 4 N HCl/AcOEt (1.5 ml) was treated with PtO_2 and hydrogenated at 50 °C under 4 atmospheres for 16 h. Catalyst was removed by filtration and the filtrate was concentrated. The residue was basified with aqueous K_2CO_3 and concentrated. The residue was diluted with $CHCl_3$–MeOH (10 : 1) and insoluble materials removed by filtration. The filtrate was concentrated and the residue chromatographed on Al_2O_3 eluting with $CHCl_3$–MeOH (10 : 1) to afford **10d** as a colorless oil (0.45 g, 91%). ^{1}H-NMR ($CDCl_3$) δ: 1.15—1.70 (14H, m), 2.23 (6H, s), 2.55—2.70 (2H, m). GC-MS *m/z*: 184 (M^+).

1-Benzotriazolyl 4-Amino-5-chloro-2-methoxybenzoate (3) A solution of 4-amino-5-chloro-2-methoxybenzoic acid (**2**, 40.3 g, 200 mmol), HOBT (27.0 g, 200 mmol) and DCC (41.2 g, 200 mmol) in DMF (400 ml) was allowed to stirred at room temperature for 3 d. After the precipitate was removed by filtration, the filtrate was concentrated and the resulting residue crystallized from AcOEt to afford **3** as a beige solid (54.1 g, 85%). ^{1}H-NMR (DMSO-d_6) δ: 3.83 (3H, s), 6.58 (1H, s), 6.81 (2H, br s), 7.35—7.90 (3H, m), 8.04 (1H, m), 8.14 (1H, dd, J=6Hz, J=1Hz). FAB-MS (Pos.) *m/z*: 319, 321 ($M^+ + 1$).

4-Amino-5-chloro-*N*-[2-(1-dimethylamino-1-cyclohexyl)ethyl]-2-methoxybenzamide (1c) A solution of **10c** (3.50 g, 20.6 mmol) in CH_2Cl_2 (10 ml) was added to a stirred suspension of **3** (7.00 g, 22.0 mmol) in CH_2Cl_2 (50 ml) at room temperature and stirred for 1 h. The reaction mixture was treated with dil. HCl and $CHCl_3$, and extracted with dil. HCl after filtration. The extract was basified with K_2CO_3 and extracted with $CHCl_3$–MeOH (10 : 1). The extract was washed with brine, dried, concentrated, and the residue chromatographed on SiO_2 eluting with $CHCl_3$–MeOH–aq. ammonia (10 : 1.5 : 0.2) then crystallized from AcOEt–hexane to afford **1c** as white crystals (6.25 g, 86%).

2-(1-Dimethylamino-1-cyclohexyl)ethyl 4-Amino-5-chloro-2-methoxybenzoate (17) 4-Dimethylaminopyridine (30 mg, 0.25 mmol) was added to a solution of **3** (490 mg, 1.5 mmol) and **7c** (237 mg, 1.4 mmol) in CH_2Cl_2 (7 ml) at room temperature and stirred for 5 h. The reaction mixture was treated with dil. HCl and AcOEt, and extracted with dil. HCl after filtration. The extract was basified with K_2CO_3 and extracted with AcOEt. The extract was washed with brine, dried, concentrated and the residue chromatographed on SiO_2 eluting with $CHCl_3$–MeOH (20 : 1) and crystallized from AcOEt–hexane to afford **17** as a white solid (335 mg, 68%).

Pharmacological Activities. Isolated LMMP of the Guinea Pig Ileum[18-20] Guinea pig ileum was divided longitudinally into segments 20 mm in length. The tissues were vertically suspended in 10 ml organ baths containing Krebs-bicarbonate solution (maintained at 37 °C in 95% O_2 + CO_2) and attached to isomeric force-displacement transducers. Platinum electrodes were placed near the top and bottom of the tissue. Transmural stimulation was carried out by rectangular pulse (3 msec, 0.1 Hz, 40—50 V) and responses to electrical stimulation were isometrically recorded under a resting tension of 1 g.

The twitch response was decreased by voltage reduction to about 50% of that at supramaximal stimulation. After a stable submaximal response was obtained, a cumulative concentration–response curve to test compound was constructed. The responses to test compounds were measured in terms of their ability to enhance the twitch response to that obtained at supramaximal voltage (100%) and are expressed as the percentage enhancement of the twitch response.

After cumulative concentration–response curves to the test compounds were constructed, the tissue were exposed to 5-MOT (5-HT_4 agonist, 10 μM) for 30 min before rechallenge with the test compound. To evaluate 5-HT_4 agonistic activity, percentage enhancement of the twitch response was calculated by subtracting the responses obtained under the 5-MOT-desensitized condition from those obtained with the test drug alone. EC_{50} and maximal response values for 5-HT_4 agonism were determined by using this concentration-response curve.

Measurement of Gastric Antral Motility[27] Under halothane anesthesia, a strain gauge transducer was sutured onto the gastric antrum to measure circular muscle contractions in male beagle dogs (9—12 kg). The dogs were fasted for 18 h before each experiment. Gastric antral motility from the transducer was measured continuously. Antral motility was quantified by determining a MI which was equivalent to the integrated area between the contractile wave and base line during a certain 15 min period. The MIs for 16 sequential 15 min periods (−60—180 min) were measured from 1—1.5 h after canned food was given. The mean of the first 4 MIs was calculated and designated as a basal MI. Test compounds were orally administrated after measurement of the 4th MI (corresponding to 2—2.5 h after feeding). Each MI of 12 sequential 15 min periods after administration was expressed as a percentage of the basal MI. All results are presented as the mean ± S.E.M.

Measurement of Gastric Emptying[28] Male beagle dogs (10—12 kg) were fasted for 18 h before each experiment. Test compounds were orally administrated 30 min before feeding. The delay of gastric emptying was induced by s.c. injection of UK14,304 (adrenaline α_2 agonist, 0.02 mg/kg) just before feeding, and a mixture of canned food (20 g/kg) and acetaminophen (50 mg/kg) were then given to each dog. Blood samples were obtained at 0, 15, 30, 45, 60, 75 and 90 min after feeding, and the plasma acetaminophen concentration was measured by the dye method with a spectrophotometer. All results are presented as the mean ± S.E.M. Statistical analysis of the data was performed by Student's *t*-test for paired data. Probability values of <0.05 were considered significant.

X-Ray Crystallography of YM-47813 The free base of YM-47813 was grown from MeOH as colorless prisms. Diffraction intensities were collected from a crystal of dimensions 0.20 × 0.15 × 0.03 mm on a Rigaku AFC7R four-circle diffractometer. Of the total of 2598 unique reflections (complete for $2\theta < 120°$), 1953 satisfied the criterion $F > 3\sigma(F)$ and only these were used in the solution and refinement of the structure. Crystal data: $C_{18}H_{28}ClN_3O_2$, M.W. = 353.89, monoclinic, space group $P2_1/c$, a = 11.433(3) Å, b = 14.053(5) Å, c = 12.816(4) Å, b = 114.22(2)°, V = 1863.3(9) Å^3, Z = 4, D_c = 1.261 g/cm^3, F_{000} = 760, CuK_α radiation, graphite-monochromated, λ = 1.54178 Å.

Structure Solution and Refinement: The structure was solved by a direct method using SAPI91, and the final refinement was done by the full-matrix least-squares method with anisotropic thermal parameters for all non-hydrogen atoms and fixed isotropic thermal parameters for all hydrogen atoms. The final R value was 0.048.

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